

AD-762 473

LOCAL IMMUNITY TO THE DIARRHEA-
INDUCING TOXIN OF THE CHOLERA VIBRIO

William Burrows

Chicago University

AD 762 473

Prepared for:

Office of Naval Research

30 June 1973

DISTRIBUTED BY:

NTIS

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va. 22151

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)

Dr. William Burrows
5724 Ellis Avenue
Chicago, Illinois 60637

2a. REPORT SECURITY CLASSIFICATION

Unclassified

2b. GROUP

3. REPORT TITLE

Local immunity to the diarrhea-inducing toxin of the cholera vibrio

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Annual Progress Report and Final Technical Report 1 May 1971 - 30 June 1973

5. AUTHOR(S) (First name, middle initial, last name)

Dr. William Burrows

6. REPORT DATE

30 June 1973

7a. TOTAL NO. OF PAGES

14

7b. NO. OF REFS

10

8a. CONTRACT OR GRANT NO.

N00014-67-A-0285-0015

b. PROJECT NO.

NR 108-924

c.

9a. ORIGINATOR'S REPORT NUMBER(S)

Number 2/N00014-67-A-0285-0015

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10. DISTRIBUTION STATEMENT

Approved for public release: distribution unlimited

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY

Office of Naval Research
Biological and Medical Sciences Division
Arlington, Va. 22217

13. ABSTRACT

Challenge infection with *V. cholerae* in the ligated ileal loop of the normal adult rabbit indicated that the unwashed loop was significantly more susceptible than the washed loop. It is suggested that this was due to the potentiating effect of mucin in the former. Such challenge of actively immunized animals in which the local immunity was a function of either antitoxic or complement-dependent vibriocidal antibody showed that both kinds of immunity were protective to ca 2 logs in the washed loop, but no significant protection was observed in the unwashed loop. This was taken to suggest the primary importance of intraluminal antibody. Comparison of these results with those of challenge with cell-free toxin in which protection appeared to be a function of tissue-contained antitoxic IIS IgA indicated that intraluminal IIS IgA had a complement-independent antibacterial activity as well as antitoxic activity.

In previously reported studies both antitoxic IgA and antitoxic-vibriocidal IgG were found at the local level in hyperimmunized animals. Extension of such studies in the present report has shown that antibody-active IgG was not found locally in animals immunized with graded doses of antigen until the serum titer rose to 1×10^4 or more. Thus while IgA appears to be of local origin and excreted into the lumen of the bowel from the lamina propria via crypt epithelial cells, local IgG seemed to be derived from serum antibody. Passive immunization experiments, based on the IV inoculation of hyperimmune serum or highly purified serum IgG, tended to substantiate this view, but no definitive evidence of the mechanism of secretion was adduced. Such passively immunized animals were immune to challenge infection in unwashed loops.

DD FORM 1473 (PAGE 1)

1 NOV 65
N 0101-807-6811

Unclassified

Security Classification

AD 762473

LOCAL IMMUNITY TO THE DIARRHEA-INDUCING

TOXIN OF THE CHOLERA VIBRIO

ANNUAL PROGRESS REPORT AND FINAL TECHNICAL REPORT

BY

WILLIAM BURROWS

30 JUNE 1973



THIS RESEARCH WAS SUPPORTED BY THE OFFICE OF NAVAL RESEARCH THROUGH
CONTRACT NO0014-67-A-0285-0015, UNDER PROJECT NUMBER NR 108-924

APPROVED FOR PUBLIC RELEASE; DISTRIBUTION UNLIMITED

REPRODUCTION IN WHOLE OR IN PART IS PERMITTED FOR ANY PURPOSE OF THE
UNITED STATES GOVERNMENT

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
U S Department of Commerce
Springfield VA 22151

UNIVERSITY OF CHICAGO
DEPARTMENT OF MICROBIOLOGY
CHICAGO, ILLINOIS 60637

Introduction

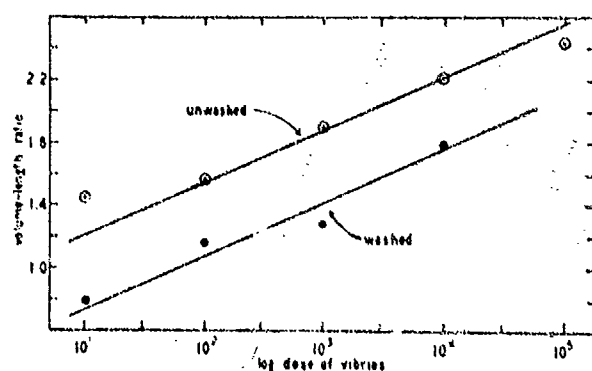
As previously indicated, the primary objective of this research has been an effective separation of the antitoxic and antibacterial facets of an effective prophylactic immunity to infection with the cholera vibrio in the rabbit ileal loop model to allow evaluation of the relative importance of the former. A separation of the antitoxic and complement-dependent vibriocidal antibody activity, the latter considered initially to represent the antibacterial response, sufficiently complete for present purposes has been achieved. Details of some aspects of the antitoxic antibody response, such as localization in the crypt cells of the lower ileum, the apparently exclusively antitoxic activity of ILS IgA, etc., have been described in Progress Reports Nos. 1 and 2 and in Technical Report No. 1.

With this foundation, it has become possible to attempt to evaluate the antitoxic element of immunity to challenge infection.

Dose-response to infection

The response of the ligated ileal loop to cell-free toxin is self-limited and dose-dependent with the elimination of time-dependency by autopsy after completion of the reaction. In contrast, that to challenge infection, although dose-dependent (Burrows and Musteikis, 1966), is not self-limited in the same sense in that the total number of vibrios increases with increasing accumulation of fluid, and the enterotoxin produced continues to increase as the reaction develops. It appeared possible, however, that the reaction to infection might be quantified with reasonable accuracy by autopsy at a constant time, 16 hours.

This possibility was investigated in an extensive series of experiments on the response to graded doses of vibrios prepared from both washed and unwashed ileum. The 569B strain of the Inaba serotype was used after passage to constant virulence by the intraluminal route. All points were derived from 30 groups of four loops with



the exception of the 1×10^1 dose of vibrios in unwashed loops for which only eight groups were available, and the 1×10^5 dose in the same series which was carried out in a single group of four loops.

It was found that log dose was linear to the volume/length (V/L) ratio measure of the response over the dose range tested. The results are summarized in Fig. 1.

Figure 1

It is apparent from these data that the correspondence of the observed points to the fitted lines for each series is close. A measure of the goodness of fit is given by the probability that the deviation of the observed from the theoretical values, $p = .4166$ and $p = .4412$ for the washed and unwashed series' respectively, i.e., essential identity. The relative precision of the titration method is illustrated by the dose of 1×10^5 vibrios in the unwashed series. As noted, the point shown in Fig. 1 was the mean of a single group of four loops. Even with this small number, the difference between the observed value and the theoretical value from the fitted line is not significant; $p = .3370$.

It is also apparent from these data that there was a marked difference, about 15-fold, in sensitivity to challenge infection between the washed and unwashed series', the latter being the more sensitive. The significance of the differences between paired points in the two series' is shown in Table 1.

Table 1

RESPONSE OF WASHED AND UNWASHED INTESTINE OF NORMAL RABBITS
TO CHALLENGE INFECTION

dose	washed	unwashed	p
1×10^1	$0.79 \pm .05$	$1.44 \pm .08$	<.0001
1×10^2	$1.16 \pm .04$	$1.56 \pm .01$	<.0001
1×10^3	$1.27 \pm .05$	$1.90 \pm .05$	<.0001
1×10^4	$1.79 \pm .05$	$2.22 \pm .02$	<.0001

These differences are clearly of sufficient magnitude that the appropriate base-line must be used in the calculation of fold-increase in vibrio dose as a measure of immunity in immunized animals in the presence (unwashed) and relative absence (washed) of intraluminal antibody. The occurrence of such differences appears to have been completely neglected in an earlier study by others (Finkelstein, 1970) whose data are not only illegitimately treated but fail to yield significant differences between immunized and normal animals (see below).

The observed, and unanticipated, differences between the two types of ileal loop preparations may have an explanation in the observations of Oza and Dutta (1970) who described the marked potentiating effect of mucin on the virulence of challenge vibrio strains, and on the toxic activity of cell-free preparations, in the infant rabbit model. It is reasonable to suppose that the latter effect is operative in the former kind of challenge since the reaction to infection is a function of formed toxin (Burrows and Mustekis, 1966). Presumably the unwashed loops described here contained mucin which was largely removed by washing, but the effect of added mucin on vibrio virulence in washed loops was not examined.

Local antibody response in the lower ileum

In any attempt to separate and evaluate the relative importance of the antibacterial and antitoxic elements of immunity to challenge infection, immunizing antigens eliciting one or the other, but not both, kinds of antibody response would appear to be required.

The development of a purely antibacterial immunity, i.e., the formation of complement-dependent vibriocidal antibody, is readily attained owing to the heat-lability of the enterotoxin by the use of boiled whole cell lysate (WCL) antigen. Its preparation has been described elsewhere (Burrows and Musteikis, 1966; Kasai and Burrows, 1966).

Although the preparation of a toxin antigen, either toxic or nontoxic, which did not induce the formation of complement-dependent vibriocidal antibody had been achieved earlier (Kaur et al, 1969), it was not as effective an antigen as impure liquid culture supernatant preparations described elsewhere (Coleman et al, 1968) in which, presumably, the impurities had an adjuvant effect. This kind of antigen, although containing no more than trace amounts of lipopolysaccharide (LPS) endotoxin does elicit the formation of complement-dependent vibriocidal antibody. This was found to be due to its content of V antigen (Kaur et al, 1969).

It was found, however, that an antitoxic, but not vibriocidal, antibody response at the local level could be produced with PSUP antigen by adjustment of dosage and immunization schedule. While hyperimmunization produced both kinds of antibody response evident as intraluminal antibody in the small bowel and in the crypt cells of intestinal epithelium in the lower ileum (Kaur et al, 1972). Grossly, two parenteral (IP) inoculations or a single intrainestinal (II) inoculation of PSUP antigen produced only antitoxic antibody in the lumen and crypt cells even though parenteral inoculation did give serum vibriocidal antibody titers of perhaps 1×10^3 to 1×10^4 . Very large doses of antigen by the II route did, however, give titratable amounts of vibriocidal antibody in wash which was associated with serum antibody titers of 1×10^7 or more. The formation of antitoxin under these conditions has been described elsewhere (Burrows et al, 1971; Kaur et al, 1971) and will not be discussed here.

On the other hand, three parenteral inoculations, or two parenteral inoculations followed by a single II inoculation did produce significant amounts of vibriocidal antibody in intestinal wash and crypt cell extracts. The occurrence of such antibody appeared to relate to serum antibody titers of 1×10^5 or more. Data illustrating the immune response to PSUP antigen as vibriocidal antibody are given in Table 2 which includes the response to both routes of inoculation and the dose-response effect.

These apparently anomalous antibody responses are associated with the antibody activity of Ig classes. As described elsewhere (Kaur et al, 1972), IIS IgA in the small bowel has antitoxic but no complement-dependent vibriocidal antibody activity, while IgG (7S) has both kinds of antibody activity. The observations described

have suggestive implications as to the mechanisms of secretion of IgG into the small bowel, and are considered further below.

Table 2
COMPLEMENT-DEPENDENT VIBRIOCIDAL ANTIBODY RESPONSE IN THE LOWER ILEUM TO PSUP ANTIGEN

dose*	route	antibody titers		crypt cells†
		serum	wash‡	
200 u	IP (2)§	1×10^3	$<1 \times 10^1$	1×10^1
200 u	IP (2)	1×10^3	$<1 \times 10^1$	5×10^1
200 u	IP (2)	$>1 \times 10^4$	1×10^1	2.5×10^1
100 u	IP (3)	$>1 \times 10^5$	5×10^1	$<1 \times 10^1$
200 u	IP (3)	$>1 \times 10^5$	5×10^1	$<1 \times 10^1$
500 u	IP (3)	$>1 \times 10^5$	1×10^2	5×10^1
200 u	IP (2)	1×10^6	1×10^3	1×10^2
1500 u	II (1)			
1500 u	II (1)	5×10^5	$<1 \times 10^1$	$<1 \times 10^1$
3000 u	II (1)	5×10^5	1×10^1	1×10^1
6000 u	II (1)	5×10^7	$<1 \times 10^1$	$<1 \times 10^1$
9000 u	II (1)	5×10^5	5×10^1	$<1 \times 10^1$

* in rabbit ileal loop units of toxin

‡ adjusted to constant volumes

† cell extracts adjusted to constant volumes

§ number of inoculations; antigen amount divided equally

Although boiled WCL antigen produced, as anticipated, no detectable antitoxic antibody in serum or in the lower ileum in response to the largest doses of antigen, 1 mg IP and 10 mg II, it was highly effective in inducing the vibriocidal antibody response. It may be noted in passing that this was undoubtedly largely anti-LPS antibody, and is perhaps to be differentiated from the anti-V antigen antibody obtained in response to the PSUP antigen; there is evidence that more than one antibody specificity may have complement-dependent vibriocidal activity (Neoh and Rowley, 1970). Serum antibody titers were uniformly high, ranging from 1×10^4 with the smaller antigen doses by the II route to 1×10^8 in the parenterally inoculated animals. Also the largest doses used in the IP series produced appreciable vibriocidal antibody in lower ileum wash and in crypt cell extracts. The kind of antibody response is illustrated by the data given in Table 3.

These antibody titers of serum, wash, and crypt cell extracts, represent the results of the titration of pooled specimens from at least four animals.

Table 3
COMPLEMENT-DEPENDENT VIBRIOCIDAL ANTIBODY RESPONSE IN THE LOWER ILEUM TO WCL ANTIGEN

dose	route	antibody titers	
		wash*	crypt cells†
0.1 mg	IP (2)‡	1×10^1	$<1 \times 10^1$
0.3 mg	IP (2)	$<1 \times 10^1$	NT
0.5 mg	IP (2)	1×10^1	$<1 \times 10^1$
1.0 mg	IP (2)	1×10^2	5×10^1
0.5 mg	II (1)	1×10^1	$<1 \times 10^1$
2.5 mg	II (1)	$<1 \times 10^1$	5×10^4
7.0 mg	II (1)	1×10^1	5×10^5
10.0 mg	II (1)	$<1 \times 10^1$	1×10^7

* adjusted to constant volumes

† cell extracts adjusted to constant volumes

‡ number of inoculations; antigen amount divided equally

Active immunity to infection challenge

Having approximated the local antibody response in the lower ileum as vibriocidal antibody in the foregoing experiments, and as antitoxin in previously published studies, extensive series of experiments have been carried out to assay the immunity to infection challenge produced by active immunization.

Two kinds of immunizing antigens were used. The crude PSUP antigen was given according to schedules previously shown to produce an antitoxic, but not complement-dependent vibriocidal, antibody response as intraluminal or crypt cell-contained antibody. This kind of crude preparation was used purposely to allow possible detection of antigens functional in immunity but not demonstrable by the usual in vitro methods. Boiled WCL antigen was used to induce the formation of vibriocidal antibody in the absence of antitoxic antibody as described above.

Unwashed and washed ileal loops of animals were challenged by the intraluminal inoculation of graded doses of vibrios in ten-fold dilutions, and the immunity assayed as the fold-increase in dose required to produce an interpolated ED_{50} dose at a V/L ratio of 1.5. The fold-increase was based on the response of normal animals to

infection as shown in Fig. 1, the increases being based on the unwashed or washed bowel response of normal animals as appropriate. Representative data for animals immunized with PSUP antigen are shown in Table 4, and for animals immunized with boiled WCL antigen in Table 5.

Table 4

ACTIVE IMMUNITY TO CHALLENGE INFECTION PRODUCED BY IMMUNIZATION
WITH PSUP ANTIGEN

immunizing antigen		unwashed	fold change		p†	wash antibody*	
dose‡	route		p†	washed		AT‡	VA¶
187	II (1)Ⓐ	457±47.2	<.0001	8.6±2.1	.1960	40.0	7
375	II (1)	134±12.8	<.0001	-1.4±0.7	.3830	11.2	<4
750	II (1)	27±2.9	<.0001	-0.4±.06	.1192	35.0	<5
1500	II (1)	343±35.3	<.0001	-1.4±.07	.3830	NT	12
100	IP (2)	0.7±2.1	.2568	-0.3±0.1	.2568	4.8	20
200	IP (2)	103±10.5	<.0001	-0.7±.03	.0320	6.7	72
400	IP (2)	117±10.9	<.0001	-6.7±1.2	.1428	13.2	84
600	IP (2)	371±36.2	<.0001	35.9±3.7	<.0001	4.1	<7

* as units per 10 cm of intestine

† probability of chance deviation from normal animals

‡ units of antitoxin

§ units of complement-dependent vibriocidal antibody

‡ number of inoculations; antigen amount divided equally

The most striking feature of these results is the good immune response, to as much as more than two logs, to challenge infection in the unwashed loops, and the failure of PSUP immunization to protect against challenge in the unwashed loop. Thus in Table 4 the only significant degree of protection was produced by the largest dose of IP antigen. The failure of the smallest dose by this route to protect in the unwashed loop is perhaps attributable to an inadequate immunizing dose of antigen. With these exceptions, the results would appear to suggest that such immunity to challenge infection is a function of intraluminal antibody (copro-antibody), but there appeared to be no regular relation between titratable antibody present in wash, given in these tables as units per 10 cm of bowel, the nominal length of the infected loop. It may also be noted that, contrary to previous observations on immunity to cell-free toxin challenge (Burrows et al, 1971), antigen given by the II route appeared to be generally as effective as that given by the IP route. This may suggest that the antitoxic element of prophylactic immunity, that considered to be tested in experiments such as these, does not play a prominent role in immunity to challenge infection.

As shown in Table 5, animals immunized with boiled WCL antigen by the IP route showed appreciable, though less marked, protection against

challenge in the unwashed, but not in the washed, loops. In contrast, the animals immunized by the II route showed not only greater protection in the unwashed loops, but also significant protection against challenge in the washed loops. Unfortunately, the data are scant on this last point, and at present writing additional experiments are under way to substantiate, or invalidate, this protection. Again there appeared to be no regular relation between protection and intraluminal complement-dependent vibriocidal antibody titers. None of the animals immunized by either route with this antigen had detectable, i.e., ≥ 5 units, of antitoxic antibody in wash.

Table 5

ACTIVE IMMUNITY TO CHALLENGE INFECTION PRODUCED BY IMMUNIZATION
WITH BOILED WCL ANTIGEN

immunizing antigen dose†	route	fold change			vibriocidal	
		unwashed	p†	washed	p† wash	antibody*
0.5	II (1)‡	-0.4±0.2	.0454	NT		31.6
2.5	II (1)	NT		14.4±3.2	<.0001	18.0
7.0	II (1)	200±21.4	<.0001	NT		34.5
10.0	II (1)	120±12.8	<.0001	55.6±8.7	<.0001	NT
0.1	IP (2)	9.1±1.9	<.0001	1.7±1.1	.1236	68.0
0.3	IP (2)	34.3±4.1	<.0001	NT		28.0
0.5	IP (2)	42.9±5.6	<.0001	-3.8±1.9	.4454	41.0
1.0	IP (2)	NT		-1.1±0.8	.1706	622.0
2.0	IP (2)	NT		6.7±2.7	.4128	NT

* as units per 10 cm of intestine

† probability of chance deviation from normal animals

‡ in milligrams

§ number of inoculations; antigen divided equally

Dose-response in protection

Examination of the data presented in Tables 4 and 5 indicates that a regular dose-response relationship to protection against challenge infection occurs in animals immunized by the IP route with either kind of antigen. This is illustrated in Fig. 2, but the figure is deceptive in that log dose refers to mg dry weight of WCL antigen, but ileal loop units of toxin in the case of PSUP. Although 1 unit of toxin is contained in 100 µg of WCL, it is destroyed by boiling, and no direct comparison of the antigenic activity measured in protection tests is possible. No such relationship is apparent in animals challenged following immunization by the II route with either antigen. It seems possible that the amount of antigen absorbed from the lumen

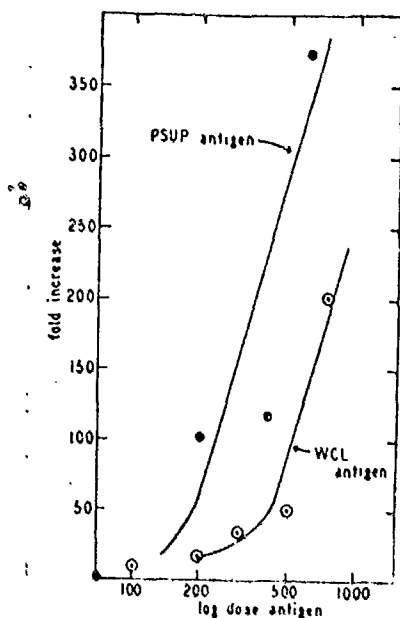


Figure 2

data allowed the calculation of the standard deviations of the means and of the difference of the means. Considering only the results of challenge with the 569B vibrio strain, that used in the present studies, which appeared to be representative, the V/L of the controls was 1.36 and that of the heterogeneous immunes 0.54. The difference, 0.82 ± 1.32 , was not significant, i.e., $p = .5352$. It cannot be considered, therefore, that the protection demonstrated here has been previously described.

Passive immunity to infection

The antibody activity functional in enteric infections, and as well in upper respiratory infections, is generally considered to be predominantly, if not exclusively, secretory IgA (11S IgA). This antibody is believed to be formed locally, in the case of the small bowel by antibody-forming cells in the lamina propria, and secreted into the lumen after the formation of the dimer and the addition, just prior to secretion, of the secretory piece.

It has been previously reported from this laboratory (Kaur et al, 1972; Technical Report No. 1) that, in addition to 11S IgA, antibody-active IgG (7S) is present but no more than a trace of IgM. The IgG has both antitoxic and complement-dependent vibriocidal antibody activity, and the 11S IgA only antitoxic activity. The apparently independent behavior of vibriocidal and antitoxic antibody, as shown for example in Table 2, suggests that the antibody-active IgG may have its origin in serum antibody, with spillover into the lumen of the bowel when the serum titer reaches levels of 1×10^4 or more. It would appear possible, therefore, to passively immunize against challenge infection, and in fact data indicating that this is the case have been described in the case of the canine model.

A small series of experiments to this end, limited by the amounts of hyperimmune sera available and the large amounts required for immunization, was carried out.

of the bowel to provide an antigenic stimulus is highly variable to account for the irregularity in the dose-response to infection.

As noted above, immunity to challenge infection in the rabbit ileal loop has been claimed to have been shown by others (Finkelstein, 1970). While the methodology in that study is somewhat obscure, a total of 13 rabbits, 6 normal and 7 immunized with varied doses of cell-free toxin antigen with and without adjuvant were used. Presumably challenge was in only a single loop with each of 6 vibrio strains and with cell-free toxin; this is inferred rather than explicitly stated in the published report. The results in immunized animals, regardless of the immunization procedure or dosage, were averaged, a highly questionable procedure, and the mean V/L ratios compared with those observed in normal animals. Assuming the validity of this procedure, the published

Animals, 3-4 to each group, were passively immunized by the IV inoculation of pooled hyperimmune serum prepared by the immunization of rabbits with WCL antigen. The total volume administered to each animal was 10 ml, and the serum contained 2000 antitoxic units per ml and had a complement-dependent vibriocidal antibody titer of 1×10^9 to 1×10^{10} . Both kinds of antibody activity present in the recipients' serum, wash, epithelial cell extract and lamina propria extract were titrated postimmunization. The animals were challenged with cell-free WCL standard toxin, and by infection at various intervals postimmunization.

The results of these experiments are shown in Tables 6, 7, and 8. The antitoxin titers are given in Table 6 and the vibriocidal antibody titers in Table 7, in both as units of antibody activity per 10 cm of lower ileum. The results of toxin and infection challenge are shown in Table 8.

Table 6
PASSIVE IMMUNITY -- ANTITOXIC ANTIBODY TITERS

hours	antitoxin titers*				
	serum at challenge	serum at autopsy	wash	cells	lamina propria
24	396	60	299	310	1415
72	476	143	10	455	1432
144	220	111	11	105	NT

* in units per 10 cm bowel

Table 7
PASSIVE IMMUNITY -- VIBRIOCIDAL ANTIBODY TITERS

hours	vibriocidal antibody titers*			
	serum†	wash	cells	lamina propria
24	$>1 \times 10^9$	2242	122	6158
48	$>1 \times 10^7$	95	87	5948
144	$>1 \times 10^6$	13	13	NT

* in units per 10 cm bowel

† at challenge

Immunity to challenge toxin appears to correlate approximately with antitoxic titers in serum and cell extracts but not, as reported earlier (Burrows et al, 1971) with intraluminal antibody titer. Immunity to challenge infection, on the other hand, failed to correlate with complement-dependent vibriocidal antibody titer in any of the materials tested, consistent with the results shown in Tables 4

and 5. Observations of the latter kind (cf Table 4), including preliminary experiments and other relatively fragmentary data not included here, have been taken to suggest the possible occurrence of antibacterial antibody activity other than that of complement-dependent vibriocidal antibody. Such a complement-independent antibacterial activity, involving the effect of antibody in preventing adsorption of vibrios to the intestinal mucosa and, in addition, a noncomplement factor supplied by the mucosal cells, has been described by Preter (1970, 1971) and may be the basis of the observations given here.

Table 8

PASSIVE IMMUNITY -- TOXIN AND INFECTION CHALLENGE

hours	toxin challenge		infection challenge	
	fold-increase	p*	fold-increase	p*
24	5.3±0.5	<.0001	77.1±7.8	<.0001
48	NT	NT	94.3±10.1	<.0001
72	5.6±0.6	<.0001	14.3±1.5	<.0001
144	1.5±0.5	.3174	NT	NT

* probability of chance deviation from normal animals

Secretion of antibody-active IgG (7S)

As noted above, antibody-active 11S IgA and IgG (7S) appear to differ with respect to their appearance in the small bowel lumen. The former occurs in the lower ileum, both as intraluminal and cell-contained antibody in consequence of local or parenteral antigenic stimulus (Tables 2 and 3; Kaur et al, 1971, 1972), while the latter, labeled differentially by its complement-dependent vibriocidal activity, tends to occur in the lower ileum only after intense antigenic stimulus (Kaur et al, 1971, 1972). Presumably these antibody classes, but not IgM, are associated with immunity to challenge in the lower ileum since IgM is present in only essentially trace amounts locally (Kaur et al, 1972).

Within the past few years considerable interest has attended to the mechanisms of 11S IgA secretion (cf Kaur et al, 1972), and it is generally believed that the dimer is formed locally and secreted after the addition of secretory piece. Nevertheless, the precise mechanism remains uncertain. In contrast, IgG is not considered to play a part in local immunity in the bowel, most workers on functional secretory antibody having assumed that observed antibody activity is solely that of 11S IgA. At present this extreme view appears to be moderating, but as yet there seems to be no information on the mechanism by which IgG arrives in the lumen of the bowel.

Since the antibody levels in the lower ileum appeared to reflect relatively high serum antibody titers of vibriocidal activity, it appeared possible that such antibody demonstrable locally might be derived from serum antibody in contrast to the locally formed IIS IgA. One experimental approach is the study of local antibody levels in passively immunized animals. The preparation of IgG (7S) from hyperimmune serum in the relatively large amounts required is practical. It consists of the 7S fraction and therefore, though considered by be IgG, may be contaminated with serum IgA monomer. Analogous preparations of IIS IgA, free of IgG and complement-dependent vibriocidal activity from extracts of intestinal tissue of immune rabbits is possible but impractical under present circumstances in the amounts required for passive immunization studies.

IgG (7S) was prepared from the same pool of hyperimmune serum used in the immediately preceding passive immunity series' by salting out the globulin and separating the IgG fraction by column chromatography. The antibody solution was adjusted to the same titers as the parent serum and given IV in 10 ml volumes to groups of animals such that there were 4 animals in each time interval group. Washes and cell and tissue extracts were pooled within these groups for antibody titration. The results of this experiment are shown in Tables 9 and 10.

Table 9

SECRETION OF ANTIBODY-ACTIVE IgG INTO THE SMALL BOWEL:
ANTITOXIC ANTIBODY

hours	serum	antitoxin titer*								
		wash			cells			tissue		
		D†	J‡	I§	D	J	I	D	J	I
24	85	16	17	21.8	<97	36	<51	585	1376	4109
48	NT	NT	NT	30	NT	NT	25	3950	1848	1856
72	NT	NT	NT	NT	1378	1461	309	NT	NT	NT

* in units per 10 cm of bowel

† duodenum.

‡ jejunum.

§ ileum

These data tend to be fragmentary because of the paucity of material, i.e., all antibody titrations could not be carried out in all cases and in the case of antitoxin the smaller amount of antitoxin titrated, the larger amount of material is required. It is apparent, however, that the IgG, both antitoxic and vibriocidal, was present in all specimens tested, with relatively larger amounts in the cell and tissue extracts than in the wash. In relating the data on antibody in wash to prior experiments, it must be borne in mind that there antibody titers of ileum alone were considered since this was the site of challenge inoculation. It is self-evident, however, that the origin of ileal intraluminal antibody is not necessarily confined to that area of the bowel, but may have been secreted at higher levels. These data suggest that secretion of IgG (7S) occurs predominantly .

in the jejunum, and to a lesser extent in the duodenum since the former titers tend to be higher in the case of vibriocidal antibody.

Table 10
SECRETION OF ANTIBODY-ACTIVE IgG INTO THE SMALL BOWEL:
VIBRIOCIDAL ANTIBODY

hours	serum	vibriocidal antibody titer*								
		wash			cells			tissue		
		D†	J‡	I¶	D	J	I	D	J	I
24	$>1 \times 10^4$	16.2	850	109	24	20	12.6	77	107	533
48	$>1 \times 10^7$	2105	18750	3000	NT	NT	3166	NT	NT	800#
72	NT	NT	119#	33.6	103#	1640#	1460#	NT	8660#	NT

* in units per 10 cm of bowel
† duodenum
‡ jejunum
¶ ileum
$\times 10^4$

The data are not definitive with regard to secretion in the ileum; since the observed titers are lower than those in the other levels of the bowel, the antibody activity may represent, at least in part, that secreted higher up but depleted en route by digestion of the immunoglobulin. The high titers of tissue extracts are notable, but the data are too scant to permit evaluation of concentration gradients; such as they are the data tend to minimize ileal secretion from the tissue into the lumen of the bowel.

Time has not permitted further study of this kind. It may be noted, however, that its vibriocidal activity serves as a sensitive label of the functionally intact IgG molecule, and is thus superior to radioactive labeling which would probably not show the lesser titers in the ileal lumen.

References

- Burrows, W., and G. M. Mustekis. 1966. Cholera infection and toxin in the rabbit ileal loop. *J. Infect. Dis.*, 116: 183-190.
- Burrows, W., J. Kaur, and L. Cercavski. 1971. The cholera enterotoxin and local immunity.
- Coleman, W. H., J. Kaur, M. E. Iwert, G. J. Kasai, and W. Burrows. 1968. Cholera toxins: purification and preliminary characterization of ileal loop reactive toxin. *J. Bacteriol.*, 96: 1137-1143.

Finkelstein, R. A. 1970. Antitoxic immunity in experimental cholera: observations with purified antigens and the ligated ileal loop model. *Infect. Immun.*, 1: 464-467.

Freter, R. 1970. Mechanism of action of intestinal antibody in experimental cholera. *Infect. Immun.*, 2: 556-562.

Freter, R. 1971. Host defense mechanisms in the intestinal tract. In: *Recent Advances in Microbiology*. Internat. Congr. Microbiol., Mexico, D. F.

Kaur, J., W. Burrows, and M. A. Furlong. 1971. Immunity to cholera: antibody response in the lower ileum of the rabbit. *J. Infect. Dis.*, 124: 359-366.

Kaur, J., J. R. McGhee, and W. Burrows. 1972. Immunity to cholera: the occurrence and nature of antibody-active immunoglobulins in the lower ileum of the rabbit. *J. Immunol.*, 108: 387-395.

Neoh, S. H., and D. Rowley. 1970. The antigens of *Vibrio cholerae* involved in the vibriocidal action of antibody and complement. *J. Infect. Dis.*, 121: 505-513.

Oza, N. B., and N. K. Dutta. 1970. A study of the pathogenesis of El Tor and classical cholera vibrios in infant rabbits. *Bull. Wld. Hlth. Org.*, 42: 642-646.